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Conformations, DNA Binding Parameters, and Antileukemic Activity of Certain Cytotoxic Protoberberine Alkaloids

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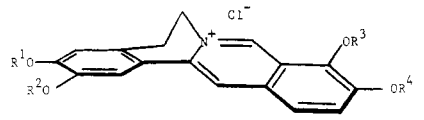
The tetrahydroprotoberberine alkaloids **5** and **7** possessing a *trans*-quinolizidine conformation display in vitro KB cytotoxicities in contrast to their corresponding diastereomers **4** and **6** which exist in the *cis*-quinolizidine conformation and are much less toxic. The DNA-binding parameters of these compounds as well as the protoberberine salts **1**, **8**, and **9** have been examined by equilibrium dialysis. Only the quaternary salts bind to DNA. The alcohol **5** showed low in vivo activity against leukemia P388 systems, while the quaternary salts **8** and **9** proved to be toxic to the host.

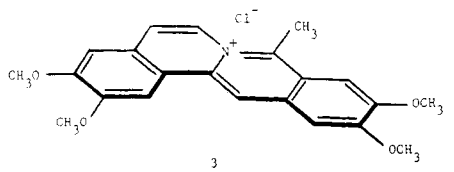
Berberine chloride (**1**) belongs to the protoberberine



1, $R^1R^2 = -CH_2-$; $R^3 = R^4 = CH_3$

2, $R^1 = R^2 = R^3 = R^4 = CH_3$

class of isoquinoline alkaloids found in a variety of plant tissues.[1,2] It antagonizes cholera toxin[3] and possesses broad spectrum antibiotic activity against Gram-positive and Gram-negative bacteria, fungi, and protozoa.[4-7] Berberine chloride (**1**) also causes the elimination of certain bacterial R factors by a hypothetical mechanism involving intercalation into closed circular superhelical plasmid DNA resulting in the formation of unnatural left-handed supercoils which cannot be replicated,[8-11] and its mutagenic action in yeast mitochondria may involve a similar mechanism.[12] Both berberine (**1**) and the structurally related natural product palmatine (**2**) are effective against experimental tumors,[13] and the synthetic dehydroprotoberberine coralyne (**3**) exhibits inhibitory activity against



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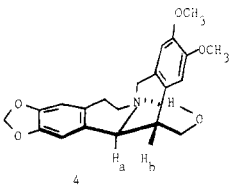
both leukemias L1210 and P388 in mice.[14-16]

A number of novel alkaloids recently became available to us during a program directed toward the development of new protoberberine syntheses.[17,18] The present communication describes observations on the in vitro cytotoxicities, conformations, DNA-binding properties, and in vivo antileukemic activities of several of these compounds.

The cytotoxicities of compounds **4**–**7** were determined



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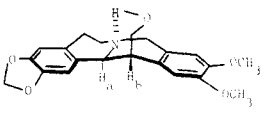

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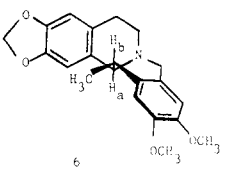

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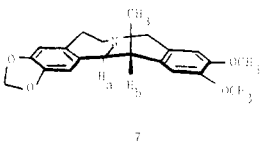

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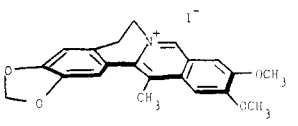

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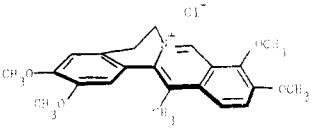

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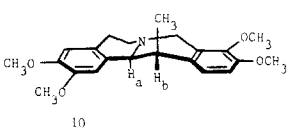

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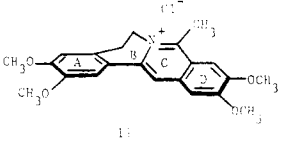

in KB cell culture (Table I). In searching for an explanation for the dramatic difference in cytotoxicities between the active compounds **5** and **7** and the inactive compounds

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Table I. In Vitro KB Cytotoxicities and DNA Intrinsic Binding Constants of Certain Protoberberine Alkaloids[a]

compd	ED_{50}, μg/mL	$K \times 10^{-4}$	DNA concn, M[b]	λ_{max}	ϵ_{max}
1		2.57 (±0.96)	6.36×10^{-3}	345	27 300
4	>100	0	6.50×10^{-3}	288	7 940
5	2.65	0	6.50×10^{-3}	288	7 590
6	>100	0	6.96×10^{-3}	290	8 110
7	0.47	0	6.96×10^{-3}	290	8 110
8	2.98	3.27 (±1.87)	6.97×10^{-3}	310	16 300
9	4.76	4.29 (±1.91)	6.97×10^{-3}	333	23 700

[a] The KB cell line, derived from a human epidermoid carcinoma,[26] was supplied by Arthur D. Little, Inc., and the cell culture screen was performed according to the standard protocol.[27] Samples were run in triplicate. The ED_{50} values were obtained by extrapolation from a least-squares fit of the dose-response curve. [b] The numbers refer to the molar concentration of nucleotide residues calculated on the basis of a molecular weight of 337 per residue.

4 and 6, our attention was directed toward the examination of their conformations. The biologically active molecules 5 and 7 were found to exist in the planar, *trans*-quinolizidine conformations, whereas in the inactive compounds 4 and 6 the quinolizidine systems are cis.[17] The biological activities of berberine (1) and coralyne (3) are thought to result from their binding to double-helical DNA by intercalation.[19-21] We therefore investigated the potential DNA-binding properties of compounds 4–7 as well as berberine (1) and the related protoberberines 8 and 13-methylpalmatine chloride (9), which were prepared by dehydrogenation of 6 and (±)-corydaline (10), respectively.

In studying the binding equilibria of small molecules with DNA, the intrinsic binding constants may be derived from the independent-site model or from the neighbor-exclusion model.[22] We quantitatively determined the binding parameters of the compounds to calf thymus DNA by equilibrium dialysis using the independent-site model.[23,24] If n is the number of binding sites per nucleotide residue, r is the ratio of bound ligands to nucleotide residues at a free ligand concentration of c moles/liter, and k is the intrinsic binding constant, then:

$$r/c = kn - kr$$

Plotting r/c vs. r (Scatchard plot, Figure 1) and calculating the slope of the steepest portion of the curve by least-squares analysis afforded the intrinsic binding constants reported in Table I. Details of the experimental procedure and conditions are reported under the Experimental Section. A detailed study by Wilson et al.[25] of the binding of berberine chloride to sonicated calf thymus DNA at pH 7.0 using the neighbor-exclusion model recently yielded a k value of 3.54×10^4.

It is apparent that an aromatic C ring is a requirement for DNA binding, and compounds 5 and 7, which do not bind to DNA, are as cytotoxic as the DNA-binding compounds 8 and 9. We are, therefore, presently unable to explain the dependence of the cytotoxicities of 4–7 on the quinolizidine conformations. We have not investigated the possibility that 5 and 7 are preferentially metabolized to 8 in the cell culture.

All of the new compounds prepared in this investigation were submitted for in vivo evaluation against P388 systems in mice (Table II). All of the compounds except 5 were inactive (T/C < 125), and those having an aromatic C ring (8 and 9) proved to be toxic to the host. The lack of antileukemic activity of 8 and 9 in P388 systems in mice is consistent with the previous observation that, in contrast to coralyne (3), which is an antileukemic agent, 5,6-dihydrocoralyne (11) does not exhibit antileukemic activity in P388 or L1210 systems.[15] The difference in antileukemic activity may be related to the fact that in 11 the plane of ring A is twisted slightly out of the plane of the CD rings, thus lowering its ability to intercalate relative to that of the completely planar coralyne (3).[25]

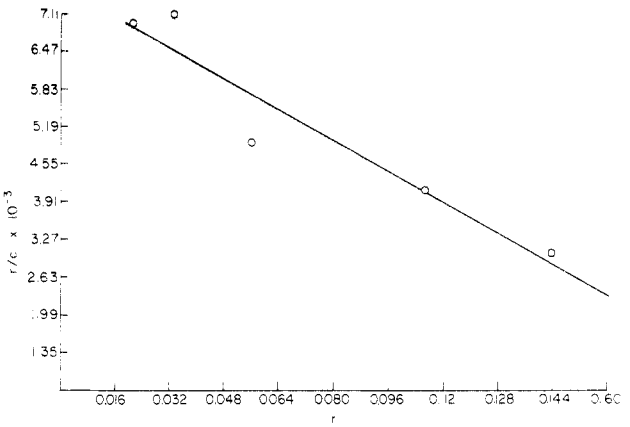

Figure 1. Binding analysis of 2,3-methylenedioxy-10,11-dimethoxy-13-methylprotoberberine iodide (8) and calf thymus DNA.

Experimental Section

All reactions were performed under a nitrogen atmosphere unless otherwise noted, and solvents were removed on a rotary evaporator under reduced pressure. Melting points were taken on a Thomas-Hoover Unimelt or a Meltemp apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-360 60-MHz instrument or JEOL PFT-100 spectrometer and, except where noted, in $CDCl_3$ solvent. Chemical shifts are reported in parts per million relative to Me_4Si as internal standard. IR spectra were recorded on a Beckman IR-33 spectrophotometer. Mass spectra were determined on a Dupont 21-492B double-focusing spectrometer using an ion source temperature of 200–280 °C, an ionization potential of 70 eV, and an ionizing current of 100 μA. Ultraviolet (UV) spectra were obtained on a Coleman Model 124 spectrometer.

Equilibrium Dialysis Experiments. The compounds listed in Table I were examined by equilibrium dialysis for their ability to bind to calf thymus DNA (Sigma Chemical Co.). The concentrations of the DNA in stock solutions were determined using an extinction coefficient $\epsilon_{260\,nm} = 6600\ M^{-1}\ cm^{-1}$. The concentrations of the protoberberine alkaloids outside the dialysis bags were determined in 1-cm light-path quartz cuvettes equipped with Teflon stoppers using the wavelengths and extinction coefficients listed in Table I. All UV spectra were measured and all equilibrium dialysis experiments were performed in duplicate in 0.005 M potassium phosphate buffer of pH 7.4 at 23 °C. Solutions were prepared using doubly distilled deionized water. All dialysis bags (Sigma Chemical Co.) were boiled in 0.005 M EDTA solution for 1 h, followed by extensive rinsing in doubly distilled water prior to use. The dialysis bags in all experiments measured 30 mm in length. The pretreated dialysis bags contained 6–7 mM solutions of DNA (1 mL), while the initial concentrations of the alkaloid solutions (25 mL) were varied from approximately 5×10^{-4} M to 5×10^{-6} M. The equilibria were established during 48 h. No binding of the alkaloids to the dialysis bags was observed, and linear Beer's law plots were obtained for the concentrations observed during the binding experiments.

2,3-Methylenedioxy-10,11-dimethoxy-13-methylprotoberberine Iodide (8). The *trans*-13-methyltetrahydroproto-

Table II. Activity of Certain Protoberberines against Leukemia P388[a]

compd	dose, mg/kg	survival	wt diff	T/C
5	400	5/5	−3.1	129
	200	5/5	−1.1	105
	100	5/5	0.1	98
	50	5/5	0.3	115
8	400	0/6	−3.5	
	200	0/6	−3.5	
	100	0/6	−3.5	
	50	3/6	−3.7	
	25	6/6	−2.2	116
	12.5	6/6	−1.7	113
	6.25	6/6	−0.3	106
9	400	0/5	−1.9	
	200	0/5	−1.9	
	100	0/5	−1.9	
	50	0/5	−1.9	
	12.5	6/6	−2.0	107
	6.25	6/6	−1.0	102
	3.13	6/6	−0.8	105

[a] For the general screening procedure and data interpretation, see R. I. Geran et al.[28] and Instruction Booklet 14, "Screening Data Summary Interpretation and Outline of Current Screen", Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., 1977.

berberine 6[17] (0.52 g, 1.4 mmol) was heated in refluxing AcOH (100 mL) containing 5% Pd/C (180 mg) for 16 h. The mixture was then filtered through Celite 545. The filtrate was evaporated, yielding a brownish yellow oil which was dissolved in 100% EtOH (10 mL). To this, 20% aqueous NaI (40 mL) was added. The precipitate was filtered and recrystallized from MeOH (25 mL), affording pure product (0.40 g, 57%): mp 257 °C dec; IR (KBr) 1495, 1460 cm^{-1}; NMR (Me_2SO-d_6) δ 9.60 (s, 1 H), 7.75 (s, 1 H), 7.50 (s, 1 H), 7.45 (s, 1 H), 7.15 (s, 1 H), 6.15 (s, 2 H), 4.60 (t, 2 H, J = 6 Hz), 4.10 (s, 3 H), 3.95 (s, 3 H), 3.15 (t, 2 H, J = 6 Hz), 2.80 (s, 3 H); mass spectrum m/e (rel intensity) 335 (M^+ − 142, 46), 334 (48), 322 (24), 142 (100), 127 (26).

13-Methylpalmatine Chloride (9). (±)-Corydaline[18] (**10**, 5.30 g, 1.43 mmol) was heated in refluxing AcOH (100 mL) containing 5% Pd/C (175 mg) for 16 h. The mixture was filtered through Celite 545, and the AcOH was evaporated from the filtrate, affording a yellow oil. The oil was dissolved in 100% EtOH (5 mL). To this solution, 20% aqueous NaCl (60 mL) was added to cause precipitation of the chloride. The product was collected by filtration, dissolved in MeOH (50 mL), and filtered. The MeOH was evaporated, and the dark yellow powder (420 mg, 73%) was dried: mp 172–174 °C dec; IR (KBr) 1585, 1505 cm^{-1}; NMR δ 10.67 (s, 1 H), 7.89 (s, 2 H), 7.16 (s, 1 H), 6.92 (s, 1 H), 5.29 (t, 2 H, J = 6 Hz), 4.35 (s, 3 H), 4.07 (s, 3 H), 4.00 (s, 3 H), 3.94 (s, 3 H), 3.24 (t, 2 H, J = 6 Hz), 2.96 (s, 3 H); mass spectrum m/e (rel intensity) 351 (M^+ − 50, 100), 350 (45), 336 (38), 322 (26), 308 (38), 292 (21), 50 (3.5).

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